DOCUMENT-IDENTIFIER: US 20030054054 A1

TITLE: Antitumor agent

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Detail Description Paragraph - DETX (20):

[0040] Insulin assays were performed using the rat insulin assay kit purchased from Crystal Chemists, Inc. (Chicago, Ill., USA). This kit uses a in an Enzyme Linked Immunosorbent Assay (non-competitive sandwich method). The assay was performed according to the manufacturer's protocol. Briefly, the standard or sample (5 .mu.l) diluted with a sample diluent was added to the wells of a multi-well polystyrene plate. The wells were precoated with antiinsulin antibody. After 2 hours of incubation, the wells were thoroughly washed with a washing buffer. A peroxidase-conjugated antiinsulin antibody solution was then added to the well. After 30 min incubation at room temperature the conjugate solution was removed and the unbound conjugate was washed off. The peroxidase substrate solution (TMB) was then added to the well and the plate was incubated at room temperature for 40 minutes. The enzyme reaction was terminated by the addition of 1N sulfuric acid at the end of this incubation period. The color was read in an microplate reader using a 405 filter.

L11 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 2

TI Highly sensitive enzyme immunoassay of proinsulin immunoreactivity with use of two monoclonal antibodies

AB A highly sensitive two-site sandwich ELISA measuring total proinsulin immunoreactive material in serum or plasma was developed. The assay was based on two **monoclonal** antibodies, an **anti-C -peptide** antibody bound to a microtest plate and a biotin-labeled anti-insulin antibody. The detection limit (3 SD above zero value) in buffer was 0.05 pmol/L, corresponding to 0.25 pmol/L in human serum (dild. 1:5). The linear calibrator range was 0.05-20 pmol/L. Interassay relative std. deviations were 4.7% at a median (range) of 2.3 pmol/L (1.4-2.8 pmol/L), 6.7% at 5.1 pmol/L (3.3-8.0 pmol/L), and 8.7% at 10.0 pmol/L (8-12 pmol/L). Mean anal. recovery of added human proinsulin (hPI) (2, 5, and 10 pmol/L) to serum was 84% (range 68-128%). Human insulin and human C-peptide did not cross-react at 5000 and 10,000 pmol/L, resp. The four major proinsulin conversion intermediates reacted 65-99%; split(32-33)hPI 74%, des(31,32)hPI 65%, split(65-66)hPI 78%, and des(64,65)hPI 99%. All serum values from 38 fasting healthy subjects were above the detection limit: median (range) 4.0 (2.1-12.6) pmol/L.

SO Clinical Chemistry (Washington, DC, United States) (1993), 39(10), 2146-50
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AU Kjems, Lise Lund; Roeder, Michael E.; Dinesen, Bo; Hartling, Svend G.; Joergensen, Peer Nobert; Binder, Christian

RBC (4) microfilm

FILE 'CAPLUS, MEDLINE, BIOSIS, CA, SCISEARCH, EMBASE' ENTERED AT 12:50:07 ON 09 JUL 2003

L1	5900	S ANTI-INSULIN OR (ANTI (W) INSULIN)
L2	63	S ANTI-C (W) PEPTIDE OR (ANTI (W) C (W) PEPTIDE)
L3	875432	S MONOCLONAL
L4	584	S L1 AND L3
L5	458	S L1 (S) L3
L6	2028245	S SOLID (W) SUPPORT OR SUBSTRATE
L7	22	S L5 AND L6
L8	6	DUPLICATE REM L7 (16 DUPLICATES REMOVED)
L9	11	S L2 AND L3
L10	11	S L2 (S) L3
L11	2	DUPLICATE REM L10 (9 DUPLICATES REMOVED)